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Patents ADP number *(if you know it)* — 7409470001

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4. Title of the invention — THERAPY AND USE OF COMPOUNDS IN THERAPY

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — ERIC POTTER CLARKSON
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THERAPY AND USE OF COMPOUNDS IN THERAPY (1)

The present invention relates to therapy and the use of compounds in therapy. In particular, it relates to the treatment and prevention of endotoxin-mediated immune activation in acute and chronic heart failure (CHF).

Chronic heart failure is a heterogeneous syndrome with an overall adverse prognosis. It is a disease in which there is a failure to pump enough blood around the body to meet its needs. Two particular predictors of adverse prognosis are neurohormonal abnormalities (Packer (1992) *J Am Coll Cardiol* **20**, 248-254) and the development of cachexia (Abel *et al* (1976) *Arch Surg* **111**, 45-50).

The syndrome of cardiac cachexia has been recognized for many centuries (Katz *et al* (1962) *Br Heart J* **24**, 257-264), but little is known about the mechanisms of the transition from heart failure to cardiac cachexia. Even the definition of cachexia and the characteristics of the cachectic patient are controversial. More than 30 years ago, the pathogenesis of cardiac cachexia was linked to dietary and metabolic factors (Pittman & Cohen (1964) *New Eng J Med* **271**, 403-409). In 1990, Levine *et al* ((1990) *New Eng J Med* **323**, 236-241) and subsequently others (McMurray *et al* (1991) *Br Heart J* **66**, 356-358; Dutka *et al* (1993) *Br Heart J* **70**, 141-143) showed the TNF-α in plasma is increased in patients with severe heart failure and coexisting cardiac cachexia, as in other wasting disorders. The plasma concentrations of TNF-α partly reflect the local tissue concentration, which is more closely related to muscle wasting (Tracey *et*

al (1990) *J Clin Invest* **86**, 2014-2024). Cytokine activation is a potential causal mechanism for the development of cachexia.

Cardiac cachectic patients suffer from loss of both muscle (ie protein reserves) and fat tissue (ie energy reserves), indicative of increased catabolism. An increased resting metabolic rate, regulated primarily by thyroid hormones (Himms-Hagen *et al* (1993) In: Grandier R. Stock, eds, Mammalian Thermogenesis, Chapman & Hall, London, UK) and catecholamines (Poehlman & Danforth (1991) *Am J Physiol* **261**, E233-E239), has been reported in CHF patients (Poehlman *et al* (1994) *Ann Intern Med* **121**, 860-862). Cortisol, another catabolic hormone, is also increased in untreated severe congested heart failure patients (Anand *et al* (1989) *Circulation* **80**, 299-305). Less is known about anabolic metabolism in heart failure. Anand *et al* ((1989) *Circulation* **80**, 299-305) found hGH to be greatly increased (≈10-fold) in untreated patients with severe heart failure. To date, these results have not been confirmed by others. Increased plasma insulin levels and insulin resistance occur in patients with CHF (Swan *et al* (1994) *Eur Heart J* **15**, 1528-1532).

The neurohormonal hypothesis (Packer (1992) *J Am Coll Cardiol* **20**, 248-254) postulates that heart failure progresses because activated endogenous neurohormonal systems exert a deleterious effect on the heart and circulation. Several studies have found neurohormonal activation to be strongly related to mortality (Cohn *et al* (1984) *New Eng J Med* **311**, 819-823; Swedberg *et al* (1990) *Circulation* **82**, 1730-1736; Francis *et al* (1993) *Circulation* **87**, (Suppl VI) VI-40 - VI-48) but different hormones correlate only weakly with each other (Swedberg *et al* (1990) *Circulation* **82**, 1730-1736). Norepinephrine and plasma renin activity were found not

to be related to peak oxygen consumption (peak VO_2) or LVEF (Francis *et al* (1993) *Circulation* **87**, (Suppl VI) VI-40-VI-48). Left ventricular function, exercise capacity, clinical status, and sympathetic activation were independently related to the progression of CHF (Francis *et al* (1993) *Circulation* **87**, (Suppl VI) VI-40-VI-48).

Anker *et al* (1997) *Circulation* **96**, 526-534 describes a study of the hormonal changes and catabolic/anabolic imbalance in CHF and concludes that cachexia is more closely associated with hormonal changes in CHF than conventional measures of the severity of CHF and suggests that the syndrome of heart failure progresses to cardiac cachexia if the normal metabolic balance between catabolism and anabolism is altered.

Anker *et al* (1997) *The Lancet* **349**, 1050-1053 suggests that the cachectic state is a strong independent risk factor for mortality in patients with CHF.

Anker *et al* (1997) *J Am Coll Cardiol* **30**, 997-1001 describes investigations of tumour necrosis factor (TNF) and steroid metabolism is CHF and concludes that there is an increase in TNF and its soluble receptor in CHF and that this increase is associated with a rise in the cortisol/DHEA (catabolic/anabolic) ratio. These changes correlate with body mass index and clinical severity of heart failure, suggesting a possible etiological link.

Anker *et al* (1997) *Am J Cardiol* **79**, 1426-1430 suggests that a chronic endotoxin challenge may cause immune activation in CHF and indicates that patients with high soluble CD14 levels have markedly increased levels

of TNF-α, soluble TNF receptors 1 and 2, and intracellular adhesion molecule-1.

Starr *et al* (1995) Direct action of endotoxin on cardiac muscle *Shock* 3(5), 380-384 suggest that endotoxin directly affects the contractile response of cardiac muscle to calcium.

Endotoxin is known to be the strongest biological stimulus for cytokine production, in particular for production of TNFα. A variety of pathophysiologic processes that directly or indirectly could contribute to deterioration of heart failure are influenced by immune activation, and specifically by TNFα:

a) TNF is detrimental for endothelial function and peripheral blood flow. In the short term TNF can up-regulate iNOS (as is seen in sepsis) and thereby contribute to vasodilation, but chronically TNF may in particular down-regulate cNOS. We have found a strong inverse correlation between the levels of TNF and the peak leg blood flow response to ischaemia ($r=-0.7$, $p<0.0001$). Impaired peripheral blood flow is closely linked to exercise capacity in CHF patients - particularly in cachectic patients.

b) Impaired peripheral blood flow is also an important component of the insulin resistance syndrome that we have shown to be present in CHF - insulin resistance appears to be a cause of energy depletion in the peripheral musculature.

c) TNF has negative inotropic effects on the heart (Starr *et al* (1995) *Shock* 3(5), 380-384.

d) The immune activation status in CHF is closely linked to the hormonal catabolic/anabolic balance in CHF patients (Anker *et al* (1997) *J Am Coll*

Cardiol **30**, 997-1001).

e) TNF is the strongest correlate of the degree of weight loss in cachectic CHF patients.

f) TNF could trigger cell apoptosis - not only in the heart, but particularly also in the periphery. This could lead to tissue dysfunction, and finally to specific and/or general tissue wasting. General wasting is then closely related to impaired prognosis in CHF.

The principal primary natural bile acids, cholic acid and chenodeoxycholic acid, are produced in the liver from cholesterol and are conjugated with glycine and taurine to give glycocholic acid, taurocholic acid, glycochenodeoxycholic acid and taurochenodeoxycholic acid before being secreted into the bile where they are present as the sodium or potassium salts (bile salts). Secondary, natural bile acids are formed in the colon by bacterial deconjugation and 7α-dehydroxylation of cholic acid and chenodeoxycholic acid producing deoxycholic acid and lithocholic acid, respectively. Ursodeoxycholic acid is a minor bile acid in man although it is the principal bile acid in bears. Dehydrocholic acid is a semi-synthetic bile acid.

The total body pool of bile salts is about 3g, and most of the secreted bile salts are reabsorbed in a process of enterohepatic recycling, so that only a small fraction of this amount must be synthesised *de novo* each day. Bile salts are strongly amphiphilic; with the acid of phospholipids they form micelles and emulsify cholesterol and other lipids in bile. Oral administration of chemodeoxycholic acid also reduces the synthesis of

cholesterol in the liver, while ursodeoxycholic acid reduces biliary cholesterol secretion apparently by increasing conversion of cholesterol to other bile acids. The bile acids (but not the bile salts) also have a choleretic action, increasing the secretion of bile, when given by mouth.

Chenodeoxycholic acid and ursodeoxycholic acid are given by mouth in the management of cholesterol-rich gallstones in patients unsuited to, or unwilling to undergo, surgery. Preparations containing bile salts have been used to assist the emulsification of fats and absorption of fat-soluble vitamins in conditions in which there is a deficiency of bile in the gastro-intestinal tract. Ox bile has also been used in the treatment of chronic constipation.

LPS binding protein is a serum protein which binds to LPS (Schumann *et al* (1990) Structure and function of lipopolysaccharide binding protein *Science* **249**, 1429-1431). The ratio of LPS to LBP may affect the immunostimulatory effects of LPS (Tobias *et al* (1997) Lipopolysaccharide binding proteins BPI and LBP form different types of complexes with LPS *J Biol Chem* **272**, 18682-18685), and the level of LBP *in vivo* can vary substantially due to transcriptional control of LBP production (Schumann *et al* (1996) Lipopolysaccharide binding protein (LBP) is a secretory class 1 acute phase protein requiring binding of the transcription factor STAT-3, C/EBPβ and AP-1 *Mol Cell Biol* **16**, 3490-3503). High concentrations of LBP may completely block LPS effects *in vitro* and in a murine sepsis model (Lamping *et al* (1998) LPS-binding protein protects mice from

septic shock caused by LPS or gram-negative bacteria *J Clin Invest* **101**, 2065-2071).

Bactericidal/permeability-increasing protein (BPI) is a protein found in human white blood cells that has multiple anti-infective and binding properties. It is capable of killing bacteria, of enhancing the effectiveness of antibiotics and of binding to and neutralising endotoxin (lipopolysaccharide; LPS). A BPI-derived pharmaceutical preparation undergoing trial is Neuprex® (Xoma Corp).

No one has previously proposed that a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule, for example LPS binding protein, BPI, bile acids or an antibody capable of binding LPS would be useful in the management of patients with either acute or chronic heart failure.

Through multiple pathways immune activation is detrimental for heart failure. We show here that endotoxin is raised in oedematous compared to non-oedematous heart failure, and propose that preventing or counteracting the presence of endotoxin or inhibiting its biological effects may lead to improved immune status, which could through multiple mechanisms improve the prognosis and clinical status of patients in the short and long term.

A first aspect of the invention provides a method of treating, preventing or ameliorating chronic heart failure or acute heart failure in a patient the method comprising administering to the patient an effective amount of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule.

A second aspect of the invention provides a method of treating, preventing or ameliorating endotoxin-mediated immune activation in acute or chronic heart failure in a patient the method comprising administering to the patient an effective amount of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule.

The following classes of patients in particular may benefit from treatment

1. Patients with acute heart failure (decompensated chronic heart failure, myocardial infarction).
2. Any decompensated heart failure patients with evidence of peripheral oedema.
3. Patients with severe heart failure (NYHA class III or IV) or with cardiac cachexia.
4. Stable CHF patients if any deterioration occurs, for example patients with a history of decompensation phases.

It is preferred that the patient has peripheral and/or bowel oedema.

Typically, in relation to the treatment of acute heart failure, the compound may be administered following myocardial infarction.

Acute heart failure is most frequently characterised by the presence of shortness of breath and oedema. It is most frequently treated by adjusting diuretics. It will be appreciated that the methods of the invention may be used in conjunction with other treatments for acute or chronic heart failure, for example treatment with diuretics. Thus, a further aspect of the invention is a method or use of the invention (as described below) wherein a diuretic is administered to the patient. The diuretic may be administered to the patient before, after or concurrently with the compound of the method or use of the invention.

It is preferred that the compound is able to substantially reduce the biological activity of endotoxin (lipopolysaccharide) such that the endotoxin has a substantially reduced effect on the liver or does not reach the liver in a substantially active form.

The compound may be, for example, a bile acid, BPI, LPS binding protein or a functional equivalent thereof or an antibody (which term includes an antibody fragment, as known to those skilled in the art) capable of binding to LPS. It will be appreciated that it is preferred that the compound is able to enter the circulation, for example following oral administration or inhalation, and is able to bind endotoxin (lipopolysaccharide; LPS) under physiological conditions in the circulation

and/or tissues of the body, for example in the blood. The ability of a compound to bind LPS may be determined as known in the art, for example using methods set out in Schumann *et al* (1990) *Science* **249**, 1429-1431.

It will be appreciated that the blood of the patient may be exposed to the said compound outside the patient's body. Thus, haemoperfusion (the passage of blood through an absorbent material) may be useful in removing LPS from blood. The blood is returned to the patient after it has been passed through the absorbent material. The absorbent material may be, for example, activated charcoal or a synthetic hydrophobic polystyrene resins that is capable of binding to endotoxin, or is capable of binding a compound as described above that is capable of binding endotoxin.

A further aspect of the invention provides a method of treating, preventing or ameliorating chronic heart failure or acute heart failure in a patient the method comprising administering to the patient an effective amount of a bile acid, BPI, LPS binding protein or a functional equivalent thereof or an antibody capable of binding to endotoxin.

A still further aspect of the invention provides a method of treating, preventing or ameliorating endotoxin-mediated immune activation in acute or chronic heart failure in a patient the method comprising administering to the patient an effective amount of a bile acid, BPI, LPS (endotoxin)

binding protein or a functional equivalent thereof or an antibody capable of binding to endotoxin.

By "bile acid" we include all naturally occurring bile acids whether from man or from another animal. Also is included bile acids which are synthetic or semi-synthetic derivatives of naturally occurring bile acids. Of course, all bile acids including those that are "naturally occurring" may be synthesised chemically.

Bile acids are available from Falk Pharma GMBH and are described, for example, in WP96/17859, DE29717252 and WO98/05339.

Bile acids for use in the method of the invention include, but are not limited to, chemodeoxycholic acid (3α, 7α - dihydroxy-5β-cholan-24-oic acid), arsodeoxycholic acid (3α, 7β-dihydroxy-5β-cholan-24-oic acid), dehydrocholic acid (3,7,12-trioxo-5β-cholan-24-oic acid), cholic acid and deoxycholic acid.

Preferably, the bile acid is a bile acid which is able to form micelles. Preferably, the bile acid is able to form a micelle around an endotoxin (lipopolysacharide molecule). It is particularly preferred that the bile acid is able to bind to endotoxin (lipopolysaccharide) molecules and substantially reduce the available endotoxin in the patient. In particular, it

is preferred if the bile acid is able to substantially reduce the biological activity of endotoxin (lipopolysaccharide) such that the endotoxin has a substantially reduced effect on the liver or does not reach the liver in a substantially active form.

It is preferred if the bile acid is any one of ursodeoxycholic acid, chemodeoxycholic acid, dehydrocholic acid, cholic acid and deoxycholic acid.

It is preferred if the bile acid is ursodeoxycholic acid.

By "LPS binding protein" is included the protein which binds to LPS (endotoxin) described in Schumann *et al* (1990) Structure and function of lipopolysaccharide binding protein *Science* **249**, 1429-1431 and fragments, variants, fusions or derivatives thereof that are capable of binding to LPS, for example as determined in Schumann *et al* (1990). Further proteins that are capable of binding to LPS are known, for example as described in US 5,760,177, isolated from horseshoe crab.

Bactericidal/permeability increasing protein (BPI) is described, for example, in Beamer *et al* (1999) The three-dimensional structure of human bactericidal/permeability-increasing protein: implications for understanding protein-lipopolysaccharide interactions *Biochem Pharmacol* 57(3), 225-9.

Antibodies that are capable of binding to endotoxin are well known to those skilled in the art, for example as described in US5,179018 (Mammalian monoclonal antibodies against endotoxin of gram-negative bacteria) and US 5,858,728 (Monoclonal antibody against LPS core).

It will be appreciated that the compound, for example bile acid, administered to the patient may be a single chemical species, for example a single chemical species of bile acid or it may be a mixture of two or more chemical species, for example two or more bile acids or a bile acid and a LPS binding protein.

The compound, for example bile acid, may be administered to the patient in any suitable form or in any suitable way. The compound or a formulation thereof may be administered by any conventional method including oral and by injection (in particular, intravascular injection). The treatment may consist of a single dose or a plurality of doses over a period of time.

Chronic use is suggested in any patient who is at increased risk of myocardial infarction (i.e. any patient with coronary artery disease - all at risk for acute heart failure) or in any patient with chronic heart failure (at risk for decompensation and cachexia development).

While it is possible for the compound, for example bile acid, BPI, LPS binding protein or antibody capable of binding to LPS (endotoxin), to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the compound, for example a bile acid, and not deleterious to the recipients thereof.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the compound (active ingredient; for example bile acid, LPS binding protein or antibody capable of binding LPS) with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be present as a bolus electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powdered or granules, optionally mixed with a binder (eg povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (eg sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyld cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

It will be appreciated that intravascular administration may be particularly desirable in the treatment of acute heart failure, for example where there is a desire for the avoidance of resorption loss of the bile acid and for a quicker onset of action.

A third aspect of the invention provides use of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule in the manufacture of a medicament for treating, preventing or ameliorating endotoxin-mediated immune activation in acute or chronic heart failure in a patient. Preferences for the said compound are as set out above. Thus, the compound may be a bile acid, LPS binding protein or an antibody capable of binding LPS.

A fourth aspect of the invention provides a pharmaceutical formulation comprising a compound as defined above and a diuretic. A further aspect of the invention provides a kit of parts useful in treating, preventing or ameliorating acute or chronic heart failure comprising a compound as defined above and a diuretic.

Suitable diuretics are known to those skilled in the art and are described, for example in Martindale The Extra Pharmacopoeia, 31st Edition.

A fifth aspect of the invention provides any novel method of treating, preventing or ameliorating acute or chronic heart failure as herein disclosed.

The invention will now be described by reference to the following Examples and Figures:

Figure 1: Plasma levels of endotoxin, TNFα and soluble CD14 in patients with chronic heart failure (CHF) with and without peripheral edema compared to healthy volunteers (mean ±standard error of the mean).

Figure 2: Effect of intensified diuretic treatment on plasma endotoxin levels in 10 CHF patients with peripheral edema (box plot displaying the 10th, 25th, 50th and 90th percentiles).

Example 1: Endotoxin and Immune Activation in Chronic Heart Failure.

Summary

Background: This study was designed to test the hypothesis that endotoxemia occurs during the congestive phase of CHF. Immune activation in chronic heart failure (CHF) patients may be secondary to endotoxin action.

Methods: We studied 20 CHF patients with recent onset of moderate to severe peripheral oedema secondary to cardiac congestion (age 64±2 y, NYHA class 3.3±0.1, mean±SEM) and compared them to 20 stable CHF patients (63±4 y, NYHA 2.6±0.2), and 14 healthy control subjects (55±4 y, ANOVA $p=0.28$). Blood samples for endotoxin measurements (LAL test, normal level <0.50 IU/mL) were collected in endotoxin free tubes. Biochemical markers of endotoxemia and inflammation, several cytokines and cell membrane proteins associated with immune activation were also measured. Ten patients were restudied within 1 week of complete resolution of oedema (5 patients survived >6 months and were restudied again).

Findings: Endotoxin levels were increased in oedematous CHF patients (0.74±0.10 IU/mL) as compared to stable CHF (0.37±0.05 IU/mL, $p=0.0009$) and controls (0.46±0.05 IU/mL, $p=0.02$); LPS binding protein (LBP) did not differ between groups. Compared to controls and stable CHF, oedematous CHF had highest levels of c-reactive protein (CRP, ANOVA $p<0.003$), tumor necrosis factor (TNF)-α ($p<0.001$), soluble (s) TNF receptor (-R)1 ($p<0.001$), sTNF-R2 ($p<0.01$), interleukin-6 ($p<0.003$), and sCD14 ($p<0.001$). Endotoxin levels

correlated with sCD14 ($r=0.30$, $p<0.03$). CRP levels correlated with procalcitonin ($r=0.74$, $p<0.0001$), TNF-α ($r=0.50$, $p=0.001$), TNF-R1 ($r=0.67$, $p<0.0001$), and TNF-R2 ($r=0.61$, $p<0.0001$). FACS analyses revealed similar CD4/8 ratios in all groups, despite significantly reduced CD4 ($p<0.02$) and elevated CD8/25 ($p<0.05$) in CHF-oedema. Diuretic treatment with resolution of oedema resulted in normalisation of endotoxin levels after 23±8 days (n=10: 0.84±0.16 to 0.45±0.07 IU/mL, $p<0.05$), but cytokines remained elevated and LBP unchanged. After freedom of oedema >3 months endotoxin levels remained stable and normal ($p=0.45$, $n=5$), and TNF-α had decreased (39.6±5.5 to 31.0±2.5 pg/mL, $p=0.079$).

Interpretation: Elevated levels of endotoxin and cytokines without a concomitant increase in LBP are found in CHF patients during an acute oedematous exacerbation. Elevated endotoxin levels are normalised by intensified diuretic treatment, whereas normalisation of TNF-α levels is delayed. These data provide evidence for a role of endotoxin as a potential cause of immune activation in patients with congestive heart failure.

The results show that LPS is raised in oedematous CHF, but normal in non-oedematous heart failure patients. The increased LPS levels are linked to raised cytokine levels. Diuretic treatment reduces LPS levels. This suggests that oedema may causally be linked with elevated LPS levels. After treating the oedema, cytokine levels (TNF etc.) but also levels of soluble CD14 (a marker of cell - LPS interaction) do not fall immediately. The cytokine levels fall only after a longer period of clinical stability. This suggests that LPS sensitivity may be abnormal in subjects

after a phase of clinical instability, i.e. despite a "normal" level of LPS the interaction with immunological cells is still intensive (sCD14 is high) and cytokine production is still increased. LPS binding protein was not increased in any patient group.

Patients with chronic heart failure (CHF) exhibit immune activation which may be related to generalised body wasting (i.e. cardiac cachexia) [1,2]. Based on the finding of increased expression of tumor necrosis factor-α (TNF-α) in cardiac tissue of CHF patients undergoing heart transplantation the failing heart itself has been suggested as the cause of immune activation [3]. To date no link between a pathogenic process and cytokine activation in heart failure has been documented, either in patients with heart failure or animal models. The precise stimulus for the increased cytokine production seen in CHF patients remains unknown.

We have previously suggested that bacterial endotoxin, lipopolysaccharide (LPS), contributes to immune activation in CHF [4]. Acute venous congestion could cause immune activation *via* several mechanisms. Regional hypoxia could facilitate the generation of oxygen free radicals and altered gut permeability may lead to bacterial or LPS translocation. Alternatively, lung infection may be present. These events may increase LPS plasma levels and trigger increased cytokine production. LPS is bound by a serum protein termed LPS binding protein (LBP) [5], and it recently has been shown that the ratio of LPS to LBP is crucial for the immunostimulatory effects of LPS [6]. LBP levels *in vivo* can vary substantially due to transcriptional activation [7]. We have recently shown that high concentrations of LBP, as seen during the acute phase response, can completely block LPS effects in vitro and in a murine sepsis model

[8]. Furthermore, in our previous study [4] patients with high soluble (s) CD14 levels (indicative of endotoxin-cell interaction and shedding of CD14 from the cell membrane [9]) showed markedly increased levels of TNF-α, sTNF receptor (R)-1 and -2, and intercellular adhesion molecule-1 (ICAM-1). A recent report documented that sCD14 alone can stimulate immune cells to produce cytokines [10].

In the present study, we measured endotoxin, LBP and sCD14 and related levels to markers of cellular and humoral immune activation in CHF patients and healthy volunteers. Among CHF patients bowel wall oedema that could cause altered gut permeability and bacterial (ie endotoxin) translocation is most likely to occur in patients with moderate to severe peripheral oedema. Thus, we compared patients with recent onset oedematous decompensation to stable non-oedematous CHF patients. In a subgroup of oedematous patients we assessed the effect of diuretic therapy, anticipating that such treatment would lead to a reduction of endotoxin.

METHODS

Fourteen healthy volunteers (age: 55±4 y) and 40 CHF patients (age: 63±3 y, $p=0.30$) were studied prospectively. The aetiology of CHF was ischaemic in 27 patients and idiopathic dilated cardiomyopathy in 13 patients. The diagnosis of CHF was based on symptomatic exercise intolerance, cardiomegaly, and documented left ventricular dysfunction (all patients had a left ventricular ejection fraction of less than 40%). No subject had clinical signs of infection, rheumatoid arthritis, or cancer. Cardiac decompensation has been associated with the presence of bowel wall oedema secondary to venous congestion. We were not able to

measure directly the degree of bowel wall oedema. The relationship between central haemodynamics and the pathophysiological alterations in CHF is weak [11,12]. In animal models there is a poor relationship between intracardiac pressures and intestinal perfusion [13]. Thus, we divided patients according to the presence or absence of a reliable marker of acute venous congestion due to cardiac failure, namely peripheral oedema.

Twenty CHF patients were clinically stable without evidence of peripheral oedema, and 20 patients presented with moderate to severe oedema to the outpatient clinic of the Royal Brompton Hospital in London, UK. The CHF patients were treated with diuretics (n=38), an angiotensin converting enzyme inhibitor (n=36), digoxin (n=14), aspirin (n=17), amiodarone (n=16) and nitrates (n=15) in varying combination. The clinical details of patients and controls are given in Table 1. Ten oedematous patients who lived close to our hospital (NYHA class IV: 5, class III: 5) were followed-up after treatment with increased doses of diuretics (increase of frusemide up to 120 mg/day, addition of bendrofluazide (2.5 or 5 mg od), and/or metolazone (5 or 10 mg od)). Of these patients three had to be admitted for 3 to 8 days for intravenous diuretic treatment. After 23 ± 8 days these patients were restudied within 1 week after complete resolution of oedema (NYHA class after treatment: III - 6, II - 4; weight loss: 3.6 ± 0.3 kg [range 2.5 to 5.0 kg]). Five patients regained clinical stability (NYHA class: III - 1, II - 4) and were restudied again 14 to 32 weeks (mean 21 ± 3 weeks) after the initial investigation when they had been free of peripheral oedema for more then 3 months. The remaining 5 patients did not reach a longer-term stable clinical state again and died 2 to 8 months after the initial investigation

without having been restudied. The research protocol was approved by the ethics committee of the Royal Brompton Hospital, and all patients and controls gave written informed consent.

Blood samples. Blood samples were collected on presentation in the outpatient clinic after supine rest for at least 15 min. An antecubital polyethylene catheter was inserted and 8 mL of venous blood were drawn into endotoxin free tubes (Endo Tube ET ®, Chromogenix AB, Sweden), and 30 mL of standard venous samples were taken for biochemical and cytokine measurements. After immediate centrifugation endotubes and plasma aliquots were stored at -80°C until analysis. In addition, 5 mL EDTA blood was taken to perform fluorescence activated cell sorting (FACS) analysis.

Assessment of endotoxin. Levels of endotoxin were measured by using a commercially available kit (Limulus Amebocyte Lysate QCL-1000 test kit, BioWhittaker Inc., Walkersville, USA). The normal level of endotoxin in this assay in healthy subjects is < 0.50 IU/mL. Endotoxin in the patient sample activates a protein in the Limulus amebocyte lysate, so that it possesses enzymatic activity. The activated enzyme catalyses the release of p-nitroaniline from a short synthetic peptide; p-nitroaniline can be detected by acidification with acetic acid, and measuring absorbance at 410 nm (sensitivity 0.03 IU/mL). The coefficient of variance for the LPS reproducibility with the LAL test kit is <10%.

Cytokine and other analyses. LBP-levels were determined by an ELISA assay as described previously [14]. Total tumor necrosis factor (TNF)-α

was measured with an ELISA test kit from Medgenix (Fleurus, Belgium; sensitivity 3.0 pg/mL; test not influenced by soluble TNF receptors). Soluble TNF receptors 1 (sTNF-R1; sensitivity 25 pg/mL), sTNF-R2 (sensitivity 2 pg/mL), and interleukin-6 (IL-6; sensitivity 0.0094 pg/mL, all kits: R&D Systems, Minneapolis, MN, USA), and sCD14 (IBL, Hamburg, Germany) were assessed by ELISA. Plasma procalcitonin (PCT) levels were measured by an immunoluminometric assay using two monoclonal antibodies (BRAHMS, Berlin, Germany) [15,16]. The normal level of PCT in this assay in healthy subjects is < 0.6 ng/ml.

FACS analysis. Whole blood samples were supplied for analysis in K-EDTA tubes (Vacutaner Systems, Falcon BD Oxford UK) and stained with fluorescently labeled monoclonal antibodies (Coulter Electronics, Luton UK) to determine peripheral lymphocyte phenotype and the proportion of CD25 receptor (CD25R) positive T cells. Briefly, a staining excess of antibody, determined by titration (data not shown), was aliquoted into 12 x 75 mm polypropylene tubes (Elkay, Hampshire UK). Two tubes were analysed for each patient sample point. The first contained control monoclonal mouse anti-human antibodies isotipically matched to the test antibodies in the second tube. The antibody-flurochrome conjugates used were CD3-PC5, CD4-FITC, CD8-ECD, CD25R-RD1. The Immunoprep formic acid lysed whole blood protocol was used in the multi-Q-prep (Coulter Electronics, Luton, UK). Lymphocyte gating was set on forward versus side scatter dot plot and compensation established by combining single colour stained leukocyte populations. Four colour flow cytometric analysis was performed on the Coulter XL-MCL employing System II software.

Statistical analyses. Normality of distribution was assessed using the Kolmogorow Smirnov test. Unpaired Student's t-test, paired t-test, ANOVA with Fisher's post hoc test, and Mann-Whitney U test were used where appropriate. Data are presented as mean ± standard error of the mean. We also performed univariate correlation analyses to establish the relationship between variables. A probability value of $p<0.05$ was considered significant.

RESULTS

Baseline analyses. In Table 1 and 2 baseline clinical characteristic and humoral measurements are detailed. Between controls and stable-CHF patients only uric acid and aspartate aminotransferase levels were significantly different. Oedematous CHF patients had more severe disease and showed a variety of biochemical abnormalities.

Endotoxin levels were highest in CHF patients with peripheral oedema (0.74±0.10 IU/mL) compared to CHF patients without oedema (0.37±0.05 IU/mL, $p=0.0009$), and controls (0.46±0.05 IU/mL, $p=0.02$) (Figure 1). Plasma levels of LBP were not statistically different between groups (stable CHF: 10.4±1.2 µg/mL, oedematous CHF: 12.1±1.3 µg/mL, controls: 9.6±1.3 µg/mL), but there was an elevated LPS / log LBP ratio in the CHF patients with oedema (oedematous CHF: 0.75±0.11, stable CHF: 0.44±0.07, controls: 0.54±0.05, ANOVA $p=0.03$, oedematous CHF vs stable CHF: $p<0.01$). In oedematous CHF patients levels were highest for CRP (+107% vs stable CHF, $p<0.03$; +252% vs controls, $p<0.001$), TNF-α (+42% vs stable CHF,

$p<0.001$; +49% vs controls, $p<0.001$, Figure 1), sTNF-R1 (+78% vs stable CHF, $p<0.006$; +171% vs controls, $p<0.0005$), sTNFR-R2 (+50% vs stable CHF, $p<0.03$; +115% vs controls, $p<0.001$), IL-6 (+241% vs stable CHF, $p<0.005$; +635% vs controls, $p<0.002$) and sCD14 (+16% vs stable CHF, $p<0.003$; +23% vs controls, $p<0.0003$, Figure 1). A trend toward increased PCT levels in oedematous CHF patients was noted (ANOVA: $p=0.073$).

Analysing the data of all subjects, there were significant correlations of sCD14 with endotoxin ($r=0.30$, $p=0.028$), as well as with TNF-α ($r=0.36$, $p=0.008$), sTNF-R1 ($r=0.46$, $p=0.0005$), and sTNF-R2 ($r=0.38$, $p<0.009$). CRP correlated with PCT ($r=0.74$, $p<0.0001$), TNF-α ($r=0.49$, $p=0.001$), sTNF-R1 ($r=0.67$, $p<0.0001$), and sTNF-R2 ($r=0.61$, $p<0.0001$), but not with endotoxin ($r=0.09$, $p=0.57$). Furthermore, PCT correlated with sTNF-R1 ($r=0.50$, $p=0.0001$) and sTNF-R2 ($r=0.53$, $p<0.0001$), but not with TNF-α ($r=0.25$, $p=0.07$) and endotoxin ($r=0.03$, $p=0.83$). There were neither simple correlations of creatinine or urea plasma levels and LPS at baseline, nor of changes of markers of kidney function over time vs the changes of LPS or cytokine concentrations over time (data not shown). Thus a bias due to latent abnormalities of kidney function seen in some oedematous patients is unlikely.

FACS analyses. There was significantly less CD4 in oedematous CHF patients (35±6%) as compared to stable-CHF (51±4%, $p<0.007$) and healthy volunteers (47±2%, $p<0.03$), whereas CD4/25 (CHF-oedema 10.6±3.3%, stable-CHF 5.5±0.7%, Con 6.7±1.1%, $p>0.2$), CD8 (CHF-

oedema 28±8%, stable-CHF 23±5%, Con 22±2%, $p>0.2$), and the CD4/8 ratio (CHF-oedema 2.6±0.9%, stable-CHF 3.3±0.8%, Con 2.5±0.3%, $p>0.2$) were not different between groups. CD8/25 was significantly higher in patients with CHF-oedema (11.6±4.0%) than in healthy volunteers (4.7±0.6%, $p<0.02$), but not stable-CHF (8.7±1.6, $p>0.2$).

Influence of diuretic treatment. Intensive diuretic treatment of CHF patients ($n=10$) resulted in weight reduction of 3.6±0.3 kg (range 2.5 to 5.0 kg), and improvement of the functional NYHA class of 9 of the 10 patients. In 8 of 10 patients a reduction of the endotoxin plasma concentration by 17 to 90% was observed (mean for all patients: -46%); the LPS levels fell from 0.84±0.16 to 0.45±0.07 IU/mL ($n=10$, $p<0.05$; Figure 2). In 2 patients with normal levels at baseline, endotoxin levels were found at the upper end of the normal range after diuretic treatment, i.e. below 0.50 IU/mL (+9% and +36% compared to baseline). Diuretic treatment did not affect plasma levels of TNF-α (baseline: 39.9±4.2 pg/mL, after: 40.2±4.1 pg/mL), sTNF-R1 (baseline: 2336±415 pg/mL, after: 2765±440 pg/mL), sTNF-R2 (baseline: 3751±378 pg/mL, after: 4029±437 pg/mL), IL-6 (baseline: 19.4±7.3 pg/mL, after: 18.3±7.6 pg/mL), sCD14 (baseline: 4474±70 ng/mL, after: 4430±241 ng/mL), or LBP (baseline: 10.3±1.2 µg/mL, after: 12.7±2.4 µg/mL) compared to baseline ($n=10$, all $p>0.20$). During further follow-up, 5 patients could be restudied when they had been free of oedema >3 months. Endotoxin remained stable at visit 3 (after 21±3 weeks: 0.49±0.03 IU/mL) compared to the second visit of these 5 patients (after

19±7 days: 0.39±0.10 IU/mL, p=0.45), but TNF-α decreased (visit 2: 39.6±5.5 vs visit 3: 31.0±2.5 pg/mL, p=0.079).

We have shown that endotoxin levels as well as pro-inflammatory cytokines are elevated in patients with heart failure who have peripheral oedema. Elevated endotoxin levels were normalised by prolonged diuretic treatment. The endotoxemia in these patients was not associated with a strong acute phase response that would have induced an increased hepatic LBP synthesis and subsequent blocking of LPS-effects. These results support the suggestion that bacterial endotoxin may be an important stimulus of immune activation in patients with chronic heart failure.

The complex of endotoxin and endotoxin binding protein activates cells *via* the CD14 protein on the surface of mononuclear phagocytes stimulating the production of TNF-α and other cytokines [17,18]. Previous studies suggested that increased sCD14 levels might be related to endotoxemia [9], but this is the first study to document directly the significant relationship between endotoxin and sCD14. Shedded and therefore soluble CD14 receptors are thought to reflect the amount of endotoxin - cell interaction over prolonged time intervals. In contrast, endotoxin itself has a short plasma half-life time (in the range of 10 to 30 min). This may explain why sCD14 levels are more closely related to the cytokine levels than endotoxin levels, as shown here and previously [4]. PCT plasma levels have been suggested to be indicative of systemic bacterial infections and are less prominent in endotoxemia [16], although the mechanisms are not clear. This study showed only a trend for raised PCT (procalcitonin) levels in oedematous CHF patients (ANOVA: $p<0.08$), and therefore only low grade bacteraemia, if at all, may be

present. That conclusion is supported by results from FACS analysis, showing only moderate changes in the pattern of cellular immune activation. Additionally, the levels of endotoxin observed in this study were well below those otherwise seen in septic shock [19]. The CHF patients studied here had no sign of active infection, and the moderate increase of plasma endotoxin levels is in keeping with the hypothesis of a translocation process. Possibly, it is endotoxin itself rather than bacteria which translocates.

Although intensified diuretic therapy resulted in normalisation of endotoxin levels, treatment did not lead immediately to reduced cytokine plasma levels, which is in keeping with a previous study [20]. This may be due to a concentration effect due to the loss of up to 5 kg body water therefore concentrating plasma levels or due to prolonged activation of monocytes/macrophages following exposure to an endotoxin stimulus during a phase of clinical deterioration with increased venous congestion, ie "normalised" endotoxin levels may still cause increased cytokine production. Indeed, such an increased cellular LPS sensitivity has recently been documented for CHF patients with acute decompensation [21], and increased TNF-α releases at baseline and after endotoxin stimulation have recently been found in cardiomyocytes from cardiac transplantation recipients, particularly for those with heart failure of ischaemic aetiology [22]. Also the previously documented raised TNF-α levels in cardiac tissue of end-stage CHF patients [3] may be due to cardiomyocytes or tissue monocytes producing increased amounts of cytokines upon stimulation by LPS, either because these patients were decompensated or because the cardiomyocytes were hypersensitive. After a prolonged phase of clinical stability TNF-α plasma levels showed a

strong trend to decrease back to normal, ie the normalisation of the relative cytokine secretion capacity may be a slow process.

Tolerance of monocytes/macrophages to endotoxin can be induced both *in vivo* and *in vitro* by endotoxin itself, and for instance it frequently occurs after severe injury [23]. One important mediator of LPS hyposensitivity is IL-10 [24]. Compared to controls, we previously found IL-10 to be lower in stable CHF patients [4]. Glucocorticoids are well known to be able to suppress LPS triggered immune activation [25], and for their general immuno suppressive effects they are considered standard in the treatment of transplant patients. Nevertheless, glucocorticoids are under certain circumstances also a prerequisite for an increased immune response [26]. In CHF patients we have recently shown that the cortisol/DHEA ratio is closely related to the degree of immune activation [27]. This marker of catabolic/anabolic balance is highest in cachectic CHF patients [2], who also demonstrate pronounced immune activation [1,2]. Increased cardiac wall stress and tissue hypoxia (both *via* local free radical generation and subsequent stimulation of the nuclear factor-kappaB pathway [28]) and hormonal catabolic/anabolic imbalance may cause immunological hypersensitivity, and endotoxin may thus be an important stimulus for cytokine production both in the heart and in the periphery. *In vitro* already low levels of LPS have detrimental effects on cardiomyocytes [29]. *In vivo* there may be a dynamic balance between heart function and immune activation in CHF patients [30]. Over time patients with frequent oedematous episodes may suffer most from the cardio-depressant [31,32] and metabolic [33,34] consequences of raised TNF-α levels, arguing for a tight control of the fluid balance of CHF patients.

In stable ambulatory patients Munger *et al* [35] have not been able to show a significant spill-over of cytokines from the heart, suggesting that cardiac production could not be the main source of the raised peripheral cytokine plasma levels. Supporting the importance of peripheral hypoxia, recently measures of increased oxidative stress have been found to correlate with sTNFR-1/2 levels [36]. We have shown that post-ischaemic peak leg blood flow in clinically stable CHF patients is inversely related to TNF-α plasma levels [37]. This may be due to a relationship between hypoxia and TNF-α production, or alternatively due to toxic effects of TNF-α on endothelial function [38]. Hypoxia *per se* may not be the most important cytokine trigger in CHF patients because of differences in the cytokine profile. Raised IL-6 plasma levels can be attributed to peripheral hypoxic conditions [39] that will certainly occur in CHF [40], but there is no report that hypoxia per se induces TNF-α, PCT, sTNF-R1 or sTNF-R2 [41]. Increased levels of soluble TNF-α receptors and particularly sCD14 are, in contrast, characteristic of endotoxin action, but not of hypoxic conditions [42].

CONCLUSION

This study demonstrates the presence of raised plasma endotoxin concentrations in patients with CHF and peripheral oedema. In the presence of unchanged levels of endotoxin binding protein this reflects a potentially pathogenic situation leading to cytokine induction. We show that normalisation of endotoxin levels can be achieved by intensified diuretic treatment. Bacterial endotoxin may be an important stimulus of immune activation in patients with chronic heart failure.

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Example 2: Experimental trials relating to the use of compounds able to bind LPS in treating chronic heart failure or acute heart failure.

Invasive assessments looking for LPS levels in different locations in the body (left and right ventricle, hepatic vein, renal vein, peripheral vein and artery, coronary sinus) may be made in patients with decompensated CHF and myocardial infarction.

This may help in confirming the source of the LPS. If LPS is highest in the hepatic vein this may indicate that the liver or more likely the bowel is the source of LPS. If LPS is higher in the hepatic vein compared to the left ventricle the lung is excluded as a source of LPS.

Gut permeability assessments may be made using sugar absorption tests in patients with and without oedema and control subjects. The precise mechanism of LPS uptake through the bowel is not clear; sugar absorption may reflect this pathway. However, kidney dysfunction (frequent in heart failure) may complicate interpretation of the results.

UDCA may be tested in patients (with oedema or with cardiac cachexia) in comparison with a placebo.

The relationship between LPS plasma levels and prognosis in oedematous and non-oedematous heart failure patients may be investigated.

Table 1: Characteristics of chronic heart failure (CHF) patients with and without peripheral edema compared to healthy volunteers.

	healthy volunteers	CHF - no edema	CHF - edema	p (ANOVA)
n	14	20	20	
age	55 ± 4	63 ± 4	64 ± 2	
NYHA class		2.6 ± 0.2	3.3 ± 0.1 ###	
weight [kg]	74 ± 7	76 ± 7	78 ± 8	
etiology: ischemic		16	11	
idiopathic dilative		4	9	
sodium [mmol/L]	139 ± 0.4	137 ± 1.2	134 ± 1.1 **	< 0.006
creatinine [µmol/L]	82 ± 4	131 ± 14	219 ± 37 *** #	< 0.003
urea [mmol/L]	5.4 ± 0.2	11.0 ± 2.0	20.0 ± 2.9 *** ##	< 0.0003
uric acid [µmol/L]	308 ± 17	417 ± 42 *	640 ± 53 *** ###	< 0.0001
ASAT [IU/L]	26 ± 3	24 ± 2	23 ± 2	
ALAT [IU/L]	23 ± 3	17 ± 1 *	14 ± 1 ##	< 0.01

Legend: *: p< 0.05, **: p<0.01, ***: p<0.001 vs healthy volunteers; #: p<0.05, ##: p<0.01, ###: p<0.001 vs no edema;
NYHA, New York Heart Association; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase

Table 2: Plasma levels of endotoxin and inflammatory markers in healthy volunteers and patients with chronic heart failure (CHF).

	healthy volunteers	CHF - no edema	CHF - edema		p (ANOVA)
endotoxin [IU/mL]	0.46 ± 0.05	0.37 ± 0.05	0.74 ± 0.10 *	###	< 0.003
TNF-α [pg/mL]	24.6 ± 2.4	25.8 ± 1.8	36.6 ± 2.8 **	##	< 0.001
sTNF-R1 [pg/mL]	708 ± 57	1077 ± 118	1922 ± 313 ***	##	< 0.001
sTNF-R2 [pg/mL]	1465 ± 264	2096 ± 330	3143 ± 388 **	#	< 0.01
sCD14 [ng/mL]	3456 ± 156	3674 ± 102	4243 ± 154 ***	##	< 0.001
procalcitonin [ng/ml]	87 ± 4	106 ± 16	145 ± 21		= 0.073
interleukin-6 [pg/mL]	2.0 ± 0.1	4.3 ± 1.2	14.7 ± 3.9 **	##	< 0.003
CRP [mg/L]	5.6 ± 0.5	9.5 ± 1.6	19.7 ± 4.6 **	#	< 0.003

Legend: *: p< 0.05, **: p<0.01, ***: p<0.001 vs healthy volunteers; #: p<0.05, ##: p<0.01, ###: p<0.001 vs no edema;
TNF, tumor necrosis factor; sTNFR, soluble TNF receptor; sCD14, soluble CD14; CRP, c-reactive protein

CLAIMS

1. A method of treating, preventing or ameliorating chronic heart failure or acute heart failure in a patient the method comprising administering to the patient an effective amount of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule.

2. A method of treating, preventing or ameliorating endotoxin-mediated immune activation in acute or chronic heart failure in a patient the method comprising administering to the patient an effective amount of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule.

3. A method according to Claim 1 or 2 wherein the compound is able to reduce the available endotoxin in the patient.

4. A method according to any one of claims 1 to 3 wherein the compound is able to substantially reduce the biological activity of endotoxin (lipopolysaccharide) such that the endotoxin has a substantially reduced effect on the liver or does not reach the liver in a substantially active form.

5. A method according to any one of claims 1 to 4 wherein the compound is a bile acid.

6. A method according to claim 5 wherein the bile acid is any one of ursodesoycholic acid, chemodeoxycholic acid, dehydrocholic acid, cholic acid and deoxycholic acid.

7. A method according to any one of claims 1 to 4 wherein the compound is LPS binding protein, bactericidal/permeability increasing protein (BPI) or an antibody capable of binding to endotoxin (lipopolysaccharide; LPS).

8. A method according to any one of the preceding claims wherein the compound is administered orally.

9. A method according to any one of Claims 1 to 7 wherein the compound is administered intravenously.

10. Use of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule in the manufacture of a medicament for treating, preventing or ameliorating chronic heart failure or acute heart failure in a patient.

11. Use of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule in the manufacture of a medicament for treating, preventing or ameliorating endotoxin-mediated immune activation in acute or chronic heart failure in a patient.

12. The use of claim 10 or claim 11 wherein the compound is a bile acid or LPS binding protein or bactericial/permeability increasing protein (BPI) or an antibody capable of binding to LPS.

13. A method of treating, preventing or ameliorating chronic heart failure or acute heart failure in a patient the method comprising administering to the patient an effective amount of a bile acid, BPI, LPS binding protein or an antibody capable of binding to LPS.

14. A method of treating, preventing or ameliorating endotoxin-mediated immune activation in acute or chronic heart failure in a patient the method comprising administering to the patient an effective amount of a bile acid, BPI, LPS binding protein or an antibody capable of binding to LPS.

15. The method or use of any of the preceding claims wherein a diuretic is administered to the patient.

16. A pharmaceutical formulation comprising bile acid or BPI or LPS binding protein or an antibody capable of binding LPS and a diuretic.

17. Any novel method of treating, preventing or ameliorating acute or chronic heart failure as herein disclosed.

18. Any novel pharmaceutical composition as herein disclosed.

ABSTRACT

THERAPY AND USE OF COMPOUNDS IN THERAPY (1)

A method of treating, preventing or ameliorating chronic heart failure or acute heart failure in a patient the method comprising administering to the patient an effective amount of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule. The compound may reduce the available endotoxin in the patient.

A method of treating, preventing or ameliorating endotoxin-mediated immune activation in acute or chronic heart failure in a patient the method comprising administering to the patient an effective amount of a compound that is able to bind to an endotoxin (lipopolysaccharide; LPS) molecule. The compound may reduce the available endotoxin in the patient.

Fig. 1

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Figure 1

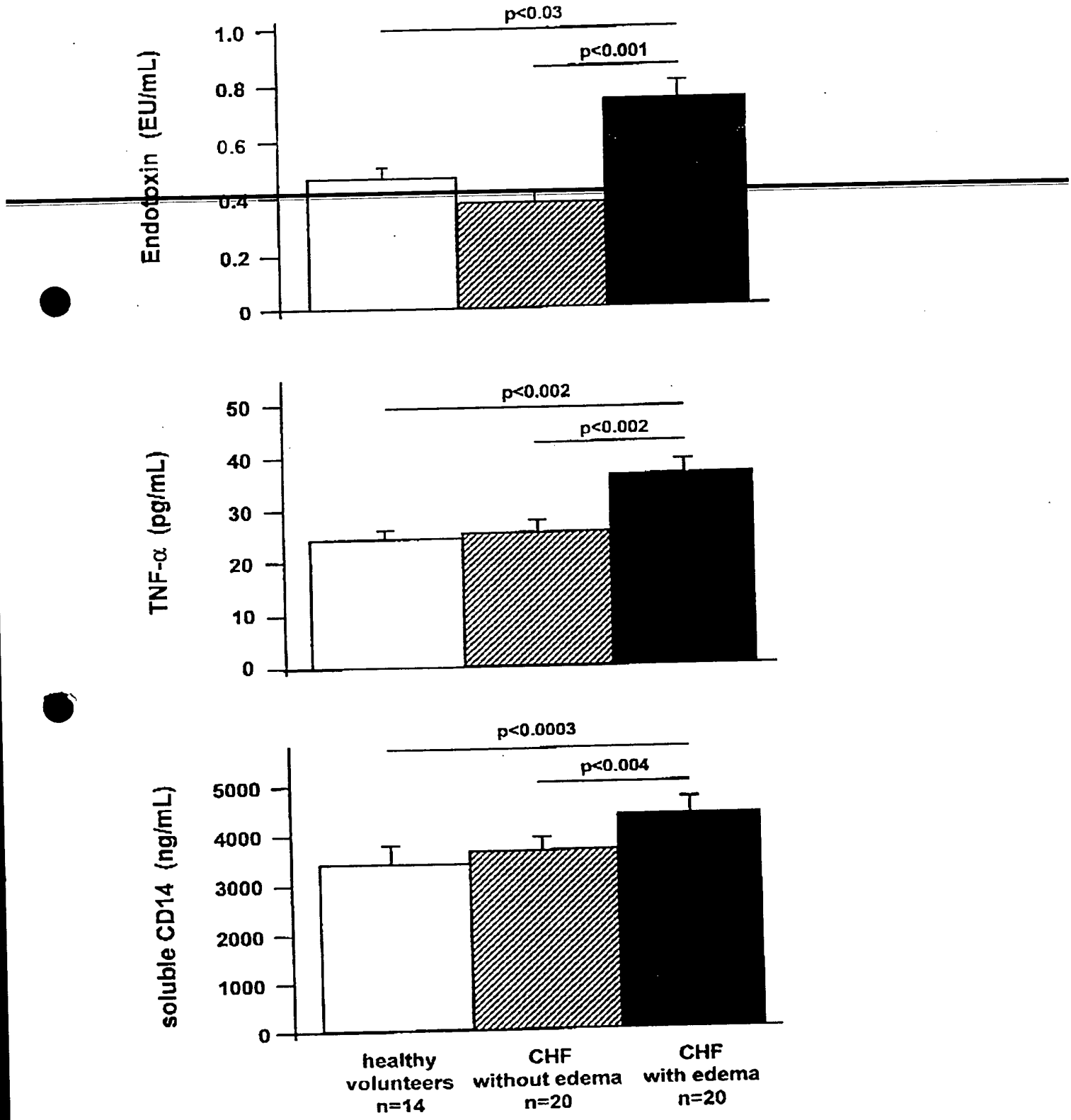
p<0.03
p<0.001
Endotoxin (EU/mL)
1.0
0.8
0.6
0.4
0.2
0
p<0.002
p<0.002
TNF-α (pg/mL)
50
40
30
20
10
0
p<0.0003
p<0.004
soluble CD14 (ng/mL)
5000
4000
3000
2000
1000
0
healthy volunteers n=14
CHF without edema n=20
CHF with edema n=20

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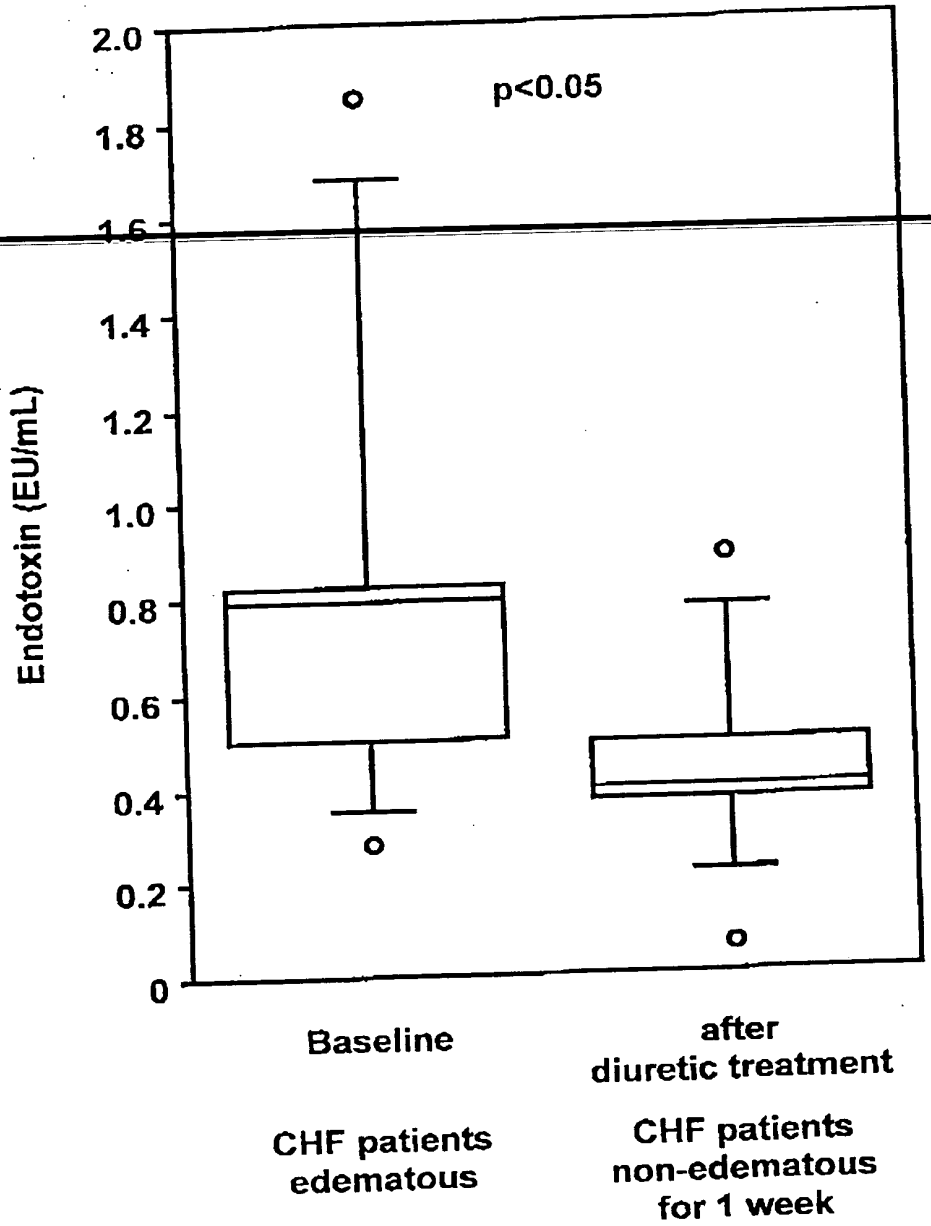
Figure 2


2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
p<0.05
Endotoxin (EU/mL)
Baseline
after diuretic treatment
CHF patients edematous
CHF patients non-edematous for 1 week